CLAIMS

1. A first method of determining the orientation of an aspherical cell in a process wherein said orientation is used in a second method to select a desired cell or part thereof from a sample of cells due to differences in size, mass, volume or density of the cell or cell part, the method being characterised in that the orientation of a cell is determined by measuring refracted non-fluorescent light provided by a phase contrast or Dark field optical system, said refracted non-fluorescent light having first been passed through one or more band pass filter(s) sufficient to exclude all wavelengths other than that remaining from the phase contrast or Dark field light source.

2. A method according to Claim 1 when used in tandem and, preferably simultaneously with, said second method to select desired cells, or parts of cells, wherein said differences in size, mass, volume or density of cells or cell parts are determined by measuring the DNA content of said cells, or parts thereof.

3. A method for determining the orientation of aspherical cells or parts of cells, according to Claim 2 which is further used in a method for selecting X chromosome-bearing sperm from Y chromosome-bearing sperm, by measuring the DNA mass of individual sperm cells.

4. A method according to any one of the preceding Claims wherein the process of selecting desired cells, or part of cells comprises the following steps:

(i) delivering suitably maintained and oriented cells, or part of cells, from a sample of cells of interest into a testing zone,
(ii) exposing said cell sample of interest to a first light source having a first wavelength,
(iii) exposing said cell sample of interest to a second light source having a second different wavelength,
(iv) collecting and measuring light energy emitted at (ii) and (iii) above via suitable detectors,
(v) analysing the light collected at (iv) to determine whether desired predetermined parameters are met,
(vi) selecting said cells, or part of said cells, which meets said desired parameters,
(vii) collecting the selected cells in a suitable viability maintenance medium, and/or
(viii) immobilising, or destroying unwanted cells, or parts of cells.

5. A method according to Claim 4 wherein, the cells to be selected are either X chromosome-bearing or Y chromosome-bearing sperm cells.

6. A method according to Claim 4 or Claim 5 wherein, the sperm cells are stained with a suitable DNA specific binding fluorochrome.

7. A method according to Claim 6 wherein, the fluorochrome is selected from SYBR green I, SYBR green II, SYBR gold, and Bisbenzimide H33342,

8. A method according to Claim 7 wherein, the first light source is an excitation light source adapted to provide fluorescent light preferably, with a peak excitation at from 488nm – 497nm.

9. A method according to any one of Claims 4 to 8 wherein, said first light source is used to fluoresce fluorochrome treated cells so as to produce sufficient fluorescence to measure the DNA content of a sperm cell.

10. A method according to Claim 9 wherein, said first light source is used to fluoresce fluorochrome treated cells so as to produce sufficient fluorescence to measure the DNA mass of a sperm cell.

11. A method according to any one of Claims 4 to 10 wherein, said second light source is used to determine the orientation of said sperm cells.

12. A method according to Claim 11 wherein, the second light source comprises part of a phase contrast or Dark field optical system and the one or more band pass filters are sufficient to exclude both the excitation and fluorescent wavelengths emitted from said first light source and any unwanted, aberrant light emitted from an immobilising or ablative means.

13. A method according to Claim 12 wherein, the cell is simultaneously exposed to said first and second light source.

14. A method according to Claim 13 wherein, sperm cells are passed through a sample orientation device adapted to hydrodynamically orient individual sperm cells such that a majority of sperm cells are presented to said first and second detectors in a uniform fashion, said orientation device being adapted to deliver viable individual sperm cells to a testing zone and wherein, said testing zone is a substantially rectangular receiving area adapted to receive and maintain the orientation of individual sperm cells for detection, DNA measurement, and either selection, immobilisation or destruction depending on one or more predetermined selection parameters.

15. A method according to Claim 14 wherein, the sperm cells to be tested are delivered to a rectangular testing zone at a flow rate above 5,000 cells per second, and preferably above 10,000 sperm cells per second.

16. A method according to Claim 15 wherein each individual sperm cell is analysed to ensure it is correctly aligned during the testing process, correct alignment indicating a true reading, and wherein

once the DNA content of each cell has been measured to determine its sex, the cells are rendered incapable of fertilisation, or not, depending on whether the individual sperm cell is of the desired chromosome content and maintained in a suitable medium.

17. An apparatus for selecting desired cells, or parts of cells, the apparatus comprising:

(i) a means for passing suitably maintained and oriented cells from a sample of cells, or parts of cells, of interest into a testing zone,

(ii) a means of exposing said cell sample of interest to a first light source having a first wavelength,

(iii) a means of exposing said cell sample of interest to a second different light source having a different wavelength,

(iv) separate detection means for collecting and, if necessary, amplifying light emitted by said sample at (ii) and (iii)

(v) a means for analysing the data collected by separate detection means (iv) to determine whether desired predetermined parameters are met,

(vi) a means for collecting, selecting and maintaining desired cells in viable condition meeting said desired predetermined parameters, and/or

(vii) an immobilising or ablative means for immobilising or eliminating unwanted cells or parts of cells not meeting said predetermined parameters.

18. An apparatus according to Claim 17 wherein, the cells or parts of cells to be tested are whole, viable sperm cells.

19. An apparatus according to Claim 18 wherein the sperm cells are delivered to a rectangular testing zone at a flow rate above 5,000 cells per second, and preferably above 10,000 sperm cells per second.

20. An apparatus according to either Claim 18 or Claim 19 wherein, the sperm cells once selected comprise a substantially homogeneously sexed population of viable sperm cells having either an X chromosome-bearing population or a Y chromosome-bearing population at a purity of greater than 95% and preferably a purity of greater than 98%.

21. An apparatus according to any one of Claims 17 to 20 wherein, said first light source is an excitation light source, preferably with a peak excitation at a wavelength of 488nm and providing a peak emission at 525nm, depending on the DNA-binding fluorochrome used.

22. An apparatus according to any one of Claims 17 to 21 said first light source is used to analyse the DNA content of a cell.

23. An apparatus according to any one of Claims 17 to 22 wherein, said second light source uses phase contrast or Dark field optics and one or more band pass filters sufficient to exclude both the excitation and fluorescence wavelengths emitted from said first light source and any unwanted, aberrant light emitted from an immobilising or ablative means

24. An apparatus according to any one of Claims 17 to 23 wherein, each individual cell is simultaneously exposed to said first and second different light sources.

25. An apparatus according to any one of Claims 17 to 24 wherein, said means for collecting light emitted from said sample after exposure to said first light source comprises part of a phase contrast or Dark field optical system.

26. An apparatus according to Claim 25 wherein, said means for collecting light emitted from said sample after exposure to said second light source is an optical detector able to collect light energy of a non-fluorescent wavelength.

27. An apparatus according to any one of Claims 17 to 26 wherein, said analysis means is a multi-channel analyser or computer programmed with suitably developed computer software.

28 An apparatus according to any one of Claims 17 to 27 wherein, the means for providing said first light source and immobilising or ablative means is a fibre optic delivery system, preferably comprising hollow cored, low OH glass fibres.

29. A delivery device adapted to sequentially deliver individual whole, viable sperm cells from a sample injection tube via a hydrodynamic radially orienting nozzle to a testing zone, a deceleration or pre-collection zone and a collection means, in a method according to any one of Claims 1-16, the delivery device comprising:
an elongated tube comprising a first end portion and a second end portion,
the first end portion comprising a nozzle,
the second end portion comprising a pre-collection or deceleration zone, and wherein,
said first and second end portions are spaced apart either side of a substantially rectangular cross-sectioned testing zone and wherein,
said first end portion comprising said nozzle has a first end and a second end, said first end being adapted to communicate with a sample injection tube to receive said sample and said second end being contiguous with

said testing zone, the nozzle being of a size and shape sufficient to maintain a majority of said sperm cells in a laminar flow at a desired hydrodynamic radial orientation, and
said second end portion comprising a pre-collection or deceleration zone is configured to convey

sperm cells to a collection means such that said cells after exiting the testing zone selected sperm cells are maintained in a viable condition suitable for use in an *in-vitro* or *in-vivo* fertilisation procedure.

30. A delivery device according to Claim 28 wherein, the pre-collection or deceleration zone is flared outwards from the substantially rectangular cross-sectioned testing zone.

31. A delivery device according to either Claim 29 or Claim 30 wherein, the pre-collection zone comprises a series of grids or groynes sufficient to decelerate individual sperm as they proceed from said testing zone to said collection means after testing.

32. A delivery device according to any one of Claims 29 to 31 wherein, in use, as the cells pass from the injection tube and into the delivery device the orientation nozzle maintains the correct orientation of a majority of the individual cells into a position which allows for each individual cell to pass through a first light source having a first wavelength and light emitted by said cell to be detected and analysed for DNA mass, and which simultaneously allows for said cell to pass through a second different light source having a second different wavelength to be detected and analysed for correct orientation.

33. A method of selecting a desired viable sperm cell suitable for *in-vivo* or *in-vitro* fertilisation, the method having the following steps:
(i) staining intact, viable sperm collected from a male mammal with a suitable DNA specific binding fluorescent dye, such that the DNA takes up the fluorescent dye uniformly,
(ii) maintaining the stained sperm in an suitable maintenance medium sufficient to maintain the sperm and/or contained DNA within the cell in a viable condition,
(iii) passing the maintenance medium containing the sperm before a suitable excitation light source causing the stained DNA to fluoresce,
(iv) passing the maintenance medium containing the sperm through both a means for measuring the fluorescence of the stained DNA and a means for detecting the orientation of the sperm,
(v) collecting light energy emitted by said sperm cell, converting the light energy into electrical signals and analysing the electrical signals via a multi-channel analyser or suitably programmed CPU,
(vi) selecting those sperm cells meeting desired predetermined criteria and
(vii) either immobilising or destroying cells, which fail to meet, desired predetermined criteria.

34. A method according to any one of Claims 1-16 or a method according to Claim 33 for producing viable sperm for use in an in-vitro fertilisation procedure.

35. A method according to any one of Claims 1-16 or a method according to Claim 33 for producing viable sperm for use in an in-vivo fertilisation procedure.

36. A viable sperm cell, the product of a method according to any one of Claims 1 to 16 or a method according to Claim 33, derived from an apparatus according to any one of Claims 17-27, or from a delivery device according to any one of Claims 29-32.

37. An Apparatus for selecting desired cells or parts of cells according to Claim 17 substantially as herein described with reference to any one of the Examples and figures 1 and 5.

38. A delivery device according to Claim 29 substantially as herein described with reference to any one of the Examples and figures 3, 4 and 6.